health.gov.au/our-work/covid-19-vaccines/getting-your-vaccination/vaccination-after-covid-19-infection
4. Nguyen KH, Huang J, Mansfield K, Corlin L, Allen JD. COVID-19 Vaccination coverage, behaviors, and intentions among adults with previous diagnosis, United States. Emerg Infect Dis. 2022;28:631–8. https://doi.org/10.3201/eid2803.211561
5. Hall VJ, Foulkes S, Saei A, Andrews N, Oguti B, Charlett A, et al.; SIREN Study Group. COVID-19 vaccine coverage in health-care workers in England and effectiveness of BNT162b2 mRNA vaccine against infection (SIREN): a prospective, multicentre, cohort study. Lancet. 2021; 397:1725–35. https://doi.org/10.1016/S0140-6736(21)00790-X
6. Our World in Data. Australia: coronavirus pandemic country profile [cited 2022 Sep 28]. https://ourworldindata.org/coronavirus/country/australia

Address for correspondence: Heather Gidding, National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases, Cnr Hawkesbury Rd and Hainsworth St, Westmead, NSW 2145, Australia; email: heather.gidding@sydney.edu.au

Presence of *Burkholderia pseudomallei* in Soil, Nigeria, 2019

Jelmer Savelkoel,[1] Rita O. Oladele,[1] Chiedozie K. Ojide, Rebecca F. Peters, Daan W. Notermans, Justina O. Makinwa, Maaike C. de Vries, Marion A.E. Sunter, Sébastien Matamoros, Nasiru Abdullahi, Uche S. Unigwe, Alani S. Akanmu, W. Joost Wiersinga,[2] Emma Birnie[2]

Author affiliations: Amsterdam UMC location, University of Amsterdam, Amsterdam, the Netherlands (J. Savelkoel, D.W. Notermans, S. Matamoros, W.J. Wiersinga, E. Birnie); Lagos University Teaching Hospital, Lagos, Nigeria (R.O. Oladele, R.F. Peters, J.O. Makinwa, A.S. Akanmu); College of Medicine University of Lagos, Lagos (R.O. Oladele, A.S. Akanmu); Ebonyi State University, Abakaliki, Nigeria (C.K. Ojide); Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Nigeria (C.K. Ojide, U.S. Unigwe); National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands (D.W. Notermans, M.C. de Vries, M.A.E. Sunter); Federal Medical Centre, Abuja, Nigeria (N. Abdullahi); University of Nigeria Teaching Hospital, Enugu, Nigeria (U.S. Unigwe)

DOI: http://doi.org/10.3201/eid2905.221138

Melioidosis, caused by the soil-dwelling bacterium *Burkholderia pseudomallei*, is predicted to be endemic in Nigeria but is only occasionally reported. This report documents the systematic identification of the presence of *B. pseudomallei* and *B. thailandensis* in the soil across multiple states in Nigeria.

The gram-negative, soil-dwelling bacterium *Burkholderia pseudomallei* is the causative agent of melioidosis, which is an important cause of lethal community-acquired sepsis throughout the tropics (*1*). Melioidosis is predicted to be endemic in Nigeria, a country with the highest estimated annual incidence, mortality, and disease burden in Africa, partly explained by its suitable environment and large population (*2*–*4*). Clinical evidence of melioidosis in Nigeria is scarce and based only on traveler-associated cases in the United Kingdom and reports from Nigeria presuming the presence of *B. pseudomallei* (*4*–*7*). This study was a collaborative effort prompted by the African Melioidosis Workshop in Lagos, Nigeria (*4*); our goal was to determine the environmental presence of *B. pseudomallei* in Nigeria. Ethics approval was obtained from the National Health Research Ethics Committee of Nigeria (approval no. NHREC/01/01/2007-26/03/2019).

We performed an environmental soil sampling study based on consensus guidelines for the identification of *B. pseudomallei* (*8*). We consulted local residents and maps to select sites associated with the occurrence of *B. pseudomallei*, as we have done previously (*9*). Using a fixed interval grid and samples taken 5 meters apart, we collected 100 soil samples per site across 8 sites in Nigeria during the rainy season in April–May 2019 (Table; Appendix, https://wwwnc.cdc.gov/EID/article/29/5/22-1138-App1.pdf). We collected a total of 800 samples in the northwestern state Kebbi, southwestern state Ogun, and southeastern states Ebonyi and Enugu. We collected soil at a depth of 65 cm and processed 10 g of soil within 7 days to enable selective enriched culture (*8*,*10*). We screened isolates by using colony morphology and, if results were suspect, used matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI Biotyper Compass v4.1 and Compass Library v10; Bruker Daltonics, https://www.bruker.com). We subjected all presumptive *B. pseudomallei* isolates to real-time multiplex PCR and performed whole-genome sequencing on 9 *B. pseudomallei* isolates and 3 *B. thailandensis* isolates by using the NextSeq 500/550 platform (Illumina, https://www.illumina.com) (Appendix). We then included

[1]These first authors contributed equally to this article.

[2]These senior authors contributed equally to this article.

Table. Site characteristics and distribution of *Burkholderia pseudomallei* at 8 sampling sites in Nigeria, 2019

Site	Location	State	Place	Site characteristics	Sample holes positive for *B. pseudomallei*
A	Southwestern	Ogun	Lufoko	Rice field, dry	0
B	Southwestern	Ogun	Ige	Rice field, dry	0
C	Northwestern	Kebbi	Birnin Kebbi*	Rice field, moist	4
D	Northwestern	Kebbi	Birnin Kebbi*	Rice field, moist	1
E	Southwestern	Ogun	Sunmoge	Cattle, grassland next to river, moist	0
F	Southeastern	Ebonyi	Abakaliki	Rice field and cassava crops, moist	38
G	Southeastern	Ebonyi	Abakaliki	Rice swamp, wet	1
H	Southeastern	Enugu	Nenwe	Rice field, moist	14

*The sampling sites in Birnin Kebbi were located 3 km apart from each other. An overview of the geographic distribution of sampling sites for *Burkholderia pseudomallei* can be found in the Appendix (https://wwwnc.cdc.gov/EID/article/29/5/22-1138-App1.pdf).

the same *B. pseudomallei* isolates in our phylogenetic comparison and used them for antimicrobial susceptibility testing (Appendix). Sequences for the samples in this study are available on the European Nucleotide Archive database (project number PRJEB54705, sample accession nos. ERS12451640–51; https://www.ebi.ac.uk/ena/browser/home).

By using the methods described, we isolated *B. pseudomallei* from 58 (7.3%) of 800 samples in 5 (62.5%) of the 8 sampling sites (Table; Appendix). We observed the highest positivity in the southeastern states, with rates as high as 38% in Ebonyi and 14% in Enugu. We also isolated the nonpathogenic *B. thailandensis* from 193 (24.1%) of 800 samples in 4 (50%) of the 8 sampling sites. Antimicrobial susceptibility of the *B. pseudomallei* isolates displayed overall sensitivity against antibiotic agents commonly used for the treatment of melioidosis, such as ceftazidime, meropenem, and trimethoprim/sulfamethoxazole (Appendix).

We conducted phylogenetic analysis of our 9 sequenced *B. pseudomallei* isolates and 13 additional genomes originating from Africa, all retrieved from the European Nucleotide Archive database. The phylogenetic tree revealed a cluster of predominantly continental Africa origin that included all of the soil isolates from Nigeria and a cluster of strains derived mainly from the Indian Ocean region (Figure). Our *B. pseudomallei* isolates did not closely match the previously sequenced traveler-associated strain from Nigeria (ERR298772) (*7*): the genome differed by 8,370 to 9,431 core single-nucleotide polymorphisms. We speculated that the higher positivity in the southeastern states reflects the relative-

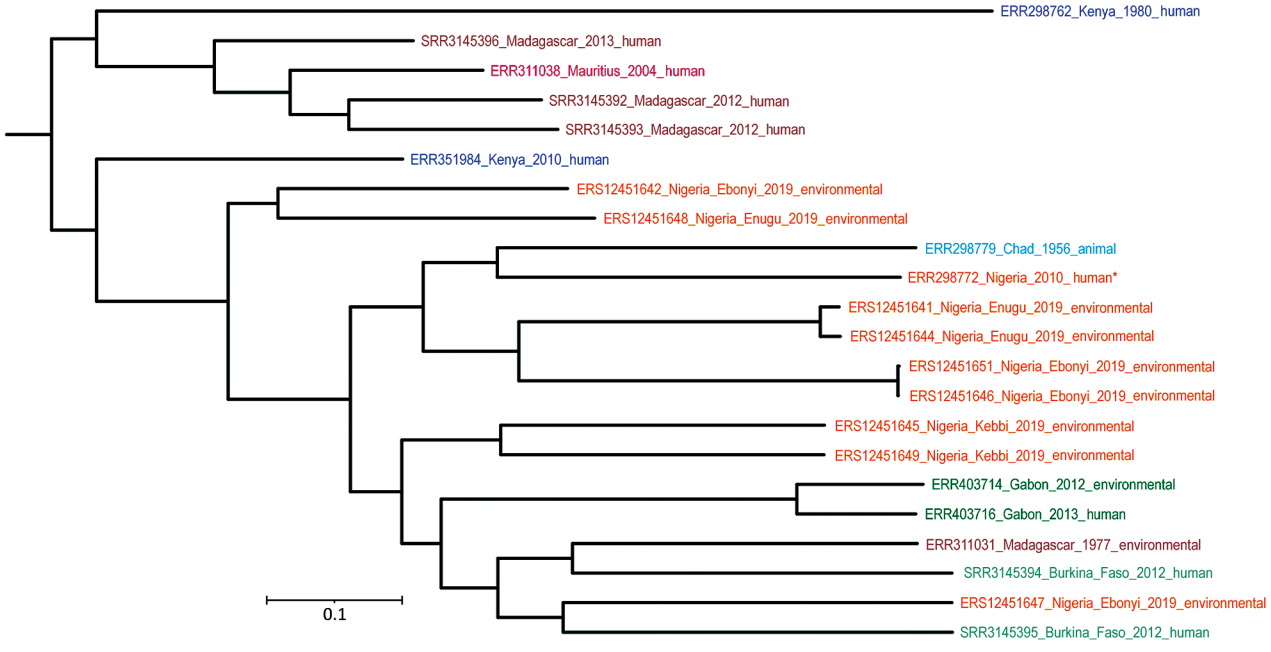


Figure. Phylogenetic tree of *Burkholderia pseudomallei* genomes from Nigeria (orange text) and additional genomes originating from Africa, all retrieved from the European Nucleotide Archive database. Tree generated by FastTree (http://www.microbesonline.org/fasttree) based on core single-nucleotide polymorphisms distance and visualized with iTOL (https://itol.embl.de). Colors indicate countries of origin. Asterisk indicates a previously sequenced, traveler-associated strain. Scale bar indicates number of nucleotide substitutions per site.

ly high annual precipitation in southeastern Nigeria as compared with sampling sites in the northwestern and southwestern states (Appendix).

Adopting a culture-based approach, combined with matrix-assisted laser desorption/ionization-time of flight mass spectrometry, real-time PCR, and whole genome sequencing allowed us to identify the environmental presence of *B. pseudomallei*. Limitations of our study include possible sampling errors and false-negative samples because we relied on a culture-based approach instead of using an additional quantitative PCR on soil samples (*9*). Moreover, we did not collect soil samples in multiple seasons to investigate a seasonal pattern, nor did we collect water or air samples.

In conclusion, we documented the systematic confirmation of the environmental presence of *B. pseudomallei* and *B. thailandensis* across multiple states in Nigeria. We identified the highest *B. pseudomallei* positivity rates in the southeastern states Ebonyi and Enugu. Phylogenetic analysis clustered our *B. pseudomallei* isolates with previous genomes that originated mostly from continental Africa. Our results highlight the probability of unrecognized melioidosis in Nigeria and warrant the attention of health workers and public health officials. Improving capacity and increasing awareness, together with environmental, serologic, and disease surveillance, is needed to increase our understanding of the melioidosis burden within Nigeria.

Acknowledgments

We thank all participants of the African Melioidosis Workshop in Lagos, Nigeria, and everyone who helped with the soil sampling. We also express our gratitude to Michelle B.S. Menig, Cornelia M. Prtenjaca Wolbers, Maaike J.C. van den Beld, and Frans A.G. Reubsaet for their expert technical assistance.

This work was supported by an Amsterdam UMC PhD Scholarship to Jelmer Savelkoel in 2021 and a research grant from the European Society of Clinical Microbiology and Infectious Diseases to Emma Birnie in 2018.

About the Author

Mr. Savelkoel is a researcher at the Center for Experimental and Molecular Medicine of Amsterdam UMC. His research interests include the global distribution and global health aspects of melioidosis.

References

1. Wiersinga WJ, Virk HS, Torres AG, Currie BJ, Peacock SJ, Dance DAB, et al. Melioidosis. Nat Rev Dis Primers. 2018;4:17107. https://doi.org/10.1038/nrdp.2017.107
2. Limmathurotsakul D, Golding N, Dance DA, Messina JP, Pigott DM, Moyes CL, et al. Predicted global distribution of *Burkholderia pseudomallei* and burden of melioidosis. Nat Microbiol. 2016;1:15008. https://doi.org/10.1038/nmicrobiol.2015.8
3. Birnie E, Virk HS, Savelkoel J, Spijker R, Bertherat E, Dance DAB, et al. Global burden of melioidosis in 2015: a systematic review and data synthesis. Lancet Infect Dis. 2019;19:892–902. https://doi.org/10.1016/S1473-3099(19)30157-4
4. Birnie E, James A, Peters F, Olajumoke M, Traore T, Bertherat E, et al. Melioidosis in Africa: time to raise awareness and build capacity for its detection, diagnosis, and treatment. Am J Trop Med Hyg. 2022;106:394–7. https://doi.org/10.4269/ajtmh.21-0673
5. Adejobi A, Ojo O, Alaka O, Odetoyin B, Onipede A. Antibiotic resistance pattern of *Pseudomonas* spp. from patients in a tertiary hospital in South-West Nigeria. Germs. 2021;11:238–45. https://doi.org/10.18683/germs.2021.1260
6. Osunla AC, Akinmolayemi AT, Makinde OA, Abioye OE, Olotu EJ, Ikuesan FA. Occurrence and antibiotics resistance signatures of *Burkholderia pseudomallei* isolated from selected hospital final effluents in Akoko metropolis within Ondo State Nigeria. Int J Public Health Res. 2021;11:1309–16.
7. Salam AP, Khan N, Malnick H, Kenna DT, Dance DA, Klein JL. Melioidosis acquired by traveler to Nigeria. Emerg Infect Dis. 2011;17:1296–8. https://doi.org/10.3201/eid1707.110502
8. Limmathurotsakul D, Dance DA, Wuthiekanun V, Kaestli M, Mayo M, Warner J, et al. Systematic review and consensus guidelines for environmental sampling of *Burkholderia pseudomallei*. PLoS Negl Trop Dis. 2013;7:e2105. https://doi.org/10.1371/journal.pntd.0002105
9. Wiersinga WJ, Birnie E, Weehuizen TA, Alabi AS, Huson MA, Huis in 't Veld RA, et al. Clinical, environmental, and serologic surveillance studies of melioidosis in Gabon, 2012–2013. Emerg Infect Dis. 2015;21:40–7. https://doi.org/10.3201/eid2101.140762
10. Manivanh L, Pierret A, Rattanavong S, Kounnavongsa O, Buisson Y, Elliott I, et al. *Burkholderia pseudomallei* in a lowland rice paddy: seasonal changes and influence of soil depth and physico-chemical properties. Sci Rep. 2017;7:3031. https://doi.org/10.1038/s41598-017-02946-z

Address for correspondence: Jelmer Savelkoel, Amsterdam UMC location University of Amsterdam, Center for Experimental and Molecular Medicine, Meibergdreef 9, 1105 AZ, Room T1.0-234, Amsterdam, the Netherlands; email: j.savelkoel@amsterdamumc.nl

Article DOI: http://doi.org/10.3201/eid2905.221138

Presence of *Burkholderia pseudomallei* in Soil, Nigeria, 2019

Appendix

Climatological Data

Appendix Table 1. Annual mean temperature and precipitation of sampled states in Nigeria (1991–2020)

Location	State	Annual mean temperature (°C)	Annual precipitation (mm)
Northwestern	Kebbi	28.71	849.46
Southwestern	Ogun	27.55	1367.16
Southeastern	Enugu	26.85	1822.48
Southeastern	Ebonyi	26.88	1966.70

Annual mean temperature and precipitation data of Nigeria (1991–2020) is derived from the Climate Change Knowledge Portal of the World Bank Group (*1*).

Polymerase Chain Reaction

We performed a TaqMan real-time multiplex polymerase chain reaction (RT-PCR) assay on all presumptive *B. pseudomallei* isolates based on colony morphology and matrix assisted laser desorption/ionization-time of flight mass spectrometry (Bruker Daltonics). We optimized a previously described RT-PCR assay with several modifications as discussed hereafter (*2*). The gene targets were as follows: *Orf11* for *B. pseudomallei*, a hypothetical 16.5 kDa protein for *B. mallei*, and *fliC* for *B. pseudomallei*, *B. mallei*, and *B. thailandensis*. The species-specific primers were modified to optimize the reaction (**Appendix Table 2**). The reaction volume of 20 µL consisted of 10 µL SensiFast master mix (Bioline), 5 µL primers-probes mix, and 5 µL of bacterial DNA. We extracted DNA of five colonies in 100 µL AE-buffer (Qiagen) at 100°C for 30 minutes. Bacterial lysates of several colonies were diluted a 100-fold with TE-buffer before being added to the final reaction volume. The primers-probes mix was prepared in TE-buffer to a final concentration of 2 pmol/µl for primers and 0.4 pmol/µl for probes. We included a positive control that consisted of a mix of *B. pseudomallei* and *B. mallei*, a no template control, and an

internal control using the nucleotide sequence of the seal herpesvirus type 1 (PhHV) to check for inhibition of the reaction (*3*). The PCR reaction was run on a LightCycler 480 II (Roche) with a denaturation cycle of 5 minutes at 95°C followed by 45 amplification cycles of 10 seconds at 95°C and 30 seconds at 60°C. An isolate was considered positive when the exponential phase was reached.

Appendix Table 2. Overview of primers and probes of the multiplex RT-PCR for *Burkholderia* species

Target species	Target gene	Nucleotide sequence	Fluorescent label
B. pseudomallei	*Orf11* forward	5′- ACA AGT GGC CCT ATG GAT TG −3′	
	Orf11 reverse	5′- TCG GTT TCG AAT AAC GGG TA −3′	
	Orf11 probe	5′- ACG ATC TCC GAG AAC GCA CTG AAC A −3′	FAM-BHQ1
B. mallei	16.5 kDa forward	5′- CGA GCT CAG CAA CCT CGT TA −3′	
	16.5 kDa reverse	5′- CGC GGT CTA CCT TGC ATA TT −3′	
	16.5 kDa probe	5′- CAG TAT CCA GGT TTC ACC GCG CTC GAC −3′	Texas Red-BHQ1
B. pseudomallei, *B. mallei*, and *B. thailandensis*	*fliC* forward	5′- GTC AAC AAI CTG CAG GCA AC −3′	
	fliC reverse	5′- CGG TTT CCT GAG IAA AGT ML −3′	
	fliC probe	5′- GGC TCG AAC AAC CTC GCG CAR G −3′	ATTO532-BHQ1
PhHV	PhHV forward	5′- GGG CGA ATC ACA GAT TGA ATC −3′	
	PhHV reverse	5′- GCG GTT CCA AAC GTA CCA A −3′	
	PhHV probe	5′- TTT TTA TGT GTC CGC CAC CAT CTG GAT C −3′	Cy5-BHQ2

Whole-Genome Sequencing

We performed whole-genome sequencing (WGS) on nine *B. pseudomallei* and three *B. thailandensis* isolates using the following methods. We extracted DNA of a loopful of bacteria in 100 µL AE-buffer at 100°C for 30 minutes. Sequences were obtained using the NextSeq 500/550 platform (Illumina). After demultiplexing, low-quality reads were discarded and adaptor sequences were trimmed using Trimmomatic v0.39 (*4*). High quality reads were used for de novo assembly using SKESA v2.4.0 (*5*). Contigs smaller than 500 bp were discarded for the following analyses. Quality of the assemblies was assessed using Quast v5.0.2 (*6*). The sequencing depth was determined by mapping the raw sequencing reads of each isolate to their respective final assembly contigs using minimap2 v2.17 (*7*), then calculating the genome coverage using SAMtools v1.14 and BEDtools v2.30.0 (*8*,*9*). SNP analysis was performed using kSNP3 v3.1.2 and visualized using FastTree v2.0 and iTOL v6 (https://itol.embl.de/) (*10–12*). Species identification was confirmed based on WGS data using the software KmerFinder v3.0.2 of the Center for Genomic Epidemiology (https://cge.food.dtu.dk/services/KmerFinder/) (*13–15*).

Based on WGS analysis, we phylogenetically characterized nine *B. pseudomallei* isolates. Additional *B. pseudomallei* genomes originating from Africa with known countries were identified via the literature and downloaded from the European Nucleotide Archive database (https://www.ebi.ac.uk/ena/browser/home) (*16*,*17*). We excluded the *B. thailandensis* isolates from further phylogenetic comparisons.

Multi Locus Sequence Typing

Multi locus sequence typing (MLST) was performed on the nine sequenced *B. pseudomallei* isolates using the software MLST v2.0.9 of the Center for Genomic Epidemiology (https://cge.food.dtu.dk/services/MLST/) (Appendix Table 3) (*21*). Raw sequencing reads were used as data input and the minimum depth for an allele was set at five times. Information on loci and gene function used in the *B. pseudomallei* MLST scheme can be found elsewhere (*22*). Next, the *B. pseudomallei* PubMLST curator and database were consulted to assign sequence types (STs) and to resolve any queries regarding imperfect matches or novel alleles (https://pubmlst.org/organisms/burkholderia-pseudomallei) (*23*).

Antimicrobial Susceptibility Testing

We performed antimicrobial susceptibility testing on the nine sequenced *B. pseudomallei* isolates following the guidelines for *B. pseudomallei* of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (*18–20*). We used the following antibiotic disks (Becton Dickinson) with disk content between parenthesis: amoxicillin-clavulanic acid (20–10 µg), ceftazidime (10 µg), imipenem (10 µg), meropenem (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), trimethoprim-sulfamethoxazole (1.25–23.75 µg), and gentamicin (10 µg). Of note: gentamicin is not included in the recommendations of EUCAST but was included as an additional control measure as we used Ashdown's selective agar for bacterial isolation. Up to four disks were placed per Mueller-Hinton (MHE) agar plate (bioMérieux). Isolates with unexpected antibiotic resistance were subjected to gradient strip testing (Liofilchem) to establish the minimal inhibitory concentration (MIC) following the previously described methods. Quality control was included as per EUCAST's instructions.

Appendix Table 3. Multi locus sequence typing of *Burkholderia pseudomallei* isolates: sequence types and allelic profiles

			Allelic profile						
Sample ID	**ENA accession number**	**ST**	**ace**	**gltB**	**gmhD**	**lepA**	**lipA**	**narK**	**ndh**
BpsC1	ERS12451645	930	1	1	3	2	5	1	1
BpsC2*	ERS12451649	12*	1	1	13	1	5	1*	1
BpsF1	ERS12451642	1720	1	1	3	2	5	2	1
BpsF2	ERS12451647	12	1	1	13	1	5	1	1
BpsF3	ERS12451651	2023	1	1	19	4	1	2	3
BpsG1	ERS12451646	2023	1	1	19	4	1	2	3
BpsH1	ERS12451644	2024	1	1	3	1	5	152	1
BpsH2*	ERS12451648	Unknown*	1	12	3	1	1	1*	1
BpsH3	ERS12451641	2026	1	1	10	1	5	149	1

*Imperfect narK hit so ST cannot be trusted. Allelic profiles of isolates with an imperfect hit were not uploaded to the PubMLST database.
Abbreviations: ENA = European Nucleotide Archive, ST = sequence type.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of sequenced *B. pseudomallei* isolates displayed overall sensitivity against antibiotic agents commonly used for treatment of melioidosis, such as ceftazidime, meropenem, and trimethoprim-sulfamethoxazole (**Appendix Table 4**). However, using the disk diffusion method unexpected antimicrobial resistance was observed against meropenem in one *B. pseudomallei* isolate. MIC testing using gradient strips did not confirm meropenem resistance as an MIC of 2 mg/L was observed (breakpoint resistance: >2 mg/L).

Appendix Table 4. Antimicrobial susceptibility of *Burkholderia pseudomallei* isolates following EUCAST's disk diffusion methods

Susceptibility testing				**EUCAST breakpoints**	
Antibiotic agent	**Disk content (µg)**	**Sensitive isolates**	**Zone diameter range (mm)**	**S ≥(mm)**	**R <(mm)**
Amoxicillin-clavulanic acid	20–10	9/9	25–30	50	22
Ceftazidime	10	9/9	22–28	50	18
Imipenem	10	9/9	31–37	29	29
Meropenem*	10	8/9*	22–31	24	24
Tetracycline*	30	9/9	26–34	23	23
Chloramphenicol	30	9/9	26–30	50	22
Trimethoprim-sulfamethoxazole	1.25–23.75	9/9	30–42	50	17
Gentamicin*	10	0/9	0–0	NA	NA

*Meropenem resistance was not confirmed using a minimal inhibitory concentration test. Tetracycline is used to screen for doxycycline susceptibility. Gentamicin is not included in EUCAST's breakpoints for *B. pseudomallei*.
Abbreviations: S = sensitive, R = resistant, NA = not applicable.

References

1. World Bank Group. Climate Change Knowledge Portal [cited 2022 7 June]. https://climateknowledgeportal.worldbank.org/country/nigeria/climate-data-historical.

2. Lowe CW, Satterfield BA, Nelson DB, Thiriot JD, Heder MJ, March JK, et al. A quadruplex real-time PCR assay for the rapid detection and differentiation of the most relevant members of the B. pseudomallei complex: B. mallei, B. pseudomallei, and B. thailandensis. PLoS One. 2016;11:e0164006. PubMed https://doi.org/10.1371/journal.pone.0164006

3. Niesters HG. Quantitation of viral load using real-time amplification techniques. Methods. 2001;25:419–29. PubMed https://doi.org/10.1006/meth.2001.1264

4. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30:2114–20. PubMed https://doi.org/10.1093/bioinformatics/btu170

5. Souvorov A, Agarwala R, Lipman DJ. SKESA: strategic k-mer extension for scrupulous assemblies. Genome Biol. 2018;19:153. PubMed https://doi.org/10.1186/s13059-018-1540-z

6. Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. Bioinformatics. 2013;29:1072–5. PubMed https://doi.org/10.1093/bioinformatics/btt086

7. Li H. Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics. 2018;34:3094–100. PubMed https://doi.org/10.1093/bioinformatics/bty191

8. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al.; 1000 Genome Project Data Processing Subgroup. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009;25:2078–9. PubMed https://doi.org/10.1093/bioinformatics/btp352

9. Quinlan AR, Hall IM. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics. 2010;26:841–2. PubMed https://doi.org/10.1093/bioinformatics/btq033

10. Gardner SN, Slezak T, Hall BG. kSNP3.0: SNP detection and phylogenetic analysis of genomes without genome alignment or reference genome. Bioinformatics. 2015;31:2877–8. PubMed https://doi.org/10.1093/bioinformatics/btv271

11. Price MN, Dehal PS, Arkin AP. FastTree 2—approximately maximum-likelihood trees for large alignments. PLoS One. 2010;5:e9490. PubMed https://doi.org/10.1371/journal.pone.0009490

12. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res. 2021;49(W1):W293–6. PubMed https://doi.org/10.1093/nar/gkab301

13. Clausen PTLC, Aarestrup FM, Lund O. Rapid and precise alignment of raw reads against redundant databases with KMA. BMC Bioinformatics. 2018;19:307. PubMed https://doi.org/10.1186/s12859-018-2336-6

14. Hasman H, Saputra D, Sicheritz-Ponten T, Lund O, Svendsen CA, Frimodt-Møller N, et al. Rapid whole-genome sequencing for detection and characterization of microorganisms directly from clinical samples. J Clin Microbiol. 2014;52:139–46. PubMed https://doi.org/10.1128/JCM.02452-13

15. Larsen MV, Cosentino S, Lukjancenko O, Saputra D, Rasmussen S, Hasman H, et al. Benchmarking of methods for genomic taxonomy. J Clin Microbiol. 2014;52:1529–39. PubMed https://doi.org/10.1128/JCM.02981-13

16. Chewapreecha C, Holden MT, Vehkala M, Välimäki N, Yang Z, Harris SR, et al. Global and regional dissemination and evolution of Burkholderia pseudomallei. Nat Microbiol. 2017;2:16263. PubMed https://doi.org/10.1038/nmicrobiol.2016.263

17. Stone NE, Hall CM, Browne AS, Sahl JW, Hutton SM, Santana-Propper E, et al. Burkholderia pseudomallei in Soil, US Virgin Islands, 2019. Emerg Infect Dis. 2020;26:2773–5. PubMed https://doi.org/10.3201/eid2611.191577

18. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0, 2022. http://www.eucast.org.

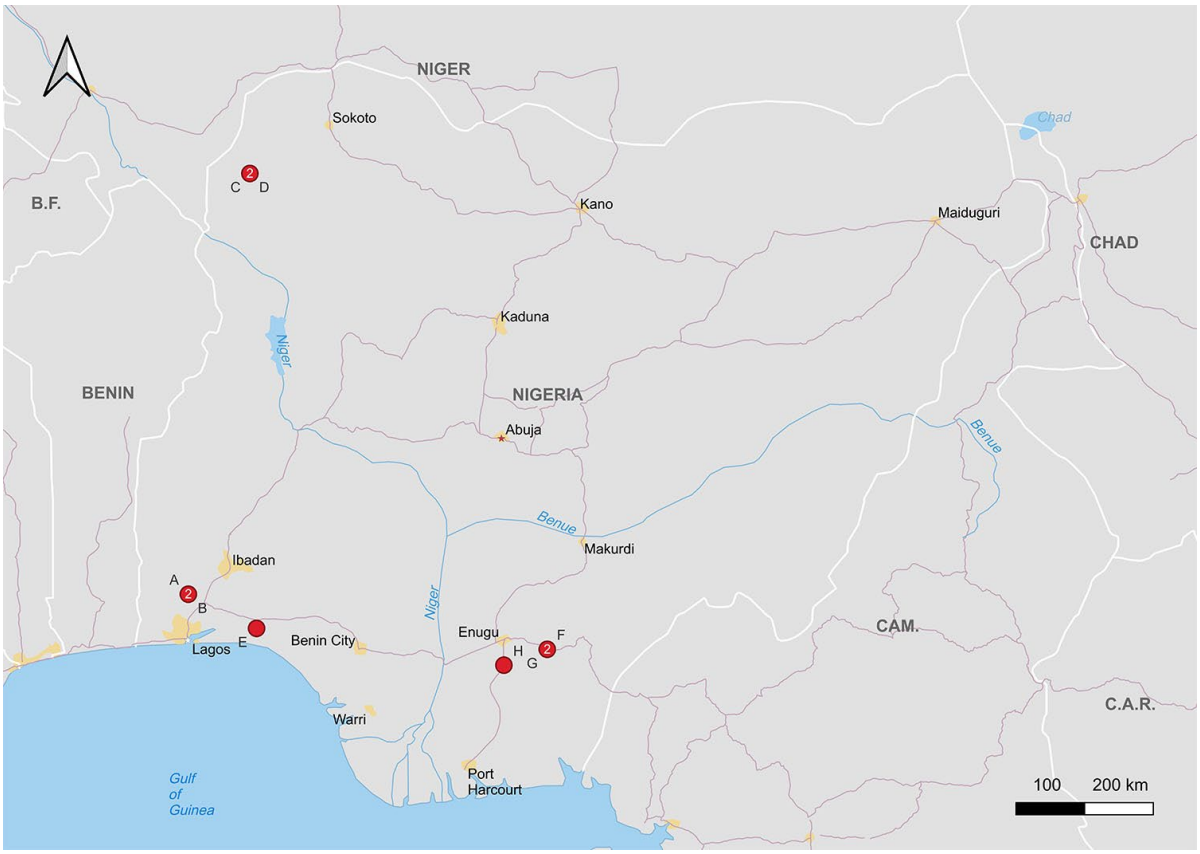
19. The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 12.0, 2022. http://www.eucast.org.

20. Dance DAB, Wuthiekanun V, Baird RW, Norton R, Limmathurotsakul D, Currie BJ. Interpreting Burkholderia pseudomallei disc diffusion susceptibility test results by the EUCAST method. Clin Microbiol Infect. 2021;27:827–9. PubMed https://doi.org/10.1016/j.cmi.2021.02.017

21. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, et al. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol. 2012;50:1355–61. PubMed https://doi.org/10.1128/JCM.06094-11

22. Godoy D, Randle G, Simpson AJ, Aanensen DM, Pitt TL, Kinoshita R, et al. Multilocus sequence typing and evolutionary relationships among the causative agents of melioidosis and glanders, Burkholderia pseudomallei and Burkholderia mallei. J Clin Microbiol. 2003;41:2068–79. PubMed https://doi.org/10.1128/JCM.41.5.2068-2079.2003

23. Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res. 2018;3:124. PubMed https://doi.org/10.12688/wellcomeopenres.14826.1



Appendix Figure. Geographic distribution of sampling sites for *Burkholderia pseudomallei* in Nigeria, 2019. Numbers indicate multiple sampling sites. Made with QGIS using Natural Earth data.